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FOREWORD

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Ann Moser 5/31/01

PI - Signature Date

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INTRODUCTION

BRCA1 was identified due to an increased risk of breast and/or ovarian cancer in females who carry a mutant allele. Recently it has been proposed that BRCA1 has a role in DNA damage repair. If this is the case, women with mutations in *BRCA1*, who have only one functional allele, may have increased susceptibility to the effects of DNA damaging agents, such as ionizing radiation. This susceptibility may result in an increased mutation rate after exposure to irradiation. Planning of prevention and treatment strategies for such high risk women must include information about environmental exposures that might increase risk. Mouse models can provide a good system in which to investigate a question such as this. We propose to test for an effect of a *Brca1* mutation on mammary tumor development in mice with an increased susceptibility to tumor formation. Mice carrying a mutation in the *Apc* gene are predisposed to mammary tumor development. The number of tumor is increased by exposure to carcinogens or irradiation. We will generate mice carrying mutant alleles at both *Brca1* and *Apc* and determine the number and time to tumor after irradiation. If heterozygosity for a mutation in *Brca1* increases the sensitivity to the effects of irradiation, we expect that the double mutant mice would develop more tumors or more advanced tumors after irradiation than the mice carrying either mutant allele alone. Specifically we propose:

1. **To identify doses of irradiation that induce mammary tumor development in 129B6 *Apc^{Min}/+* female mice.**
2. **To determine the effect of heterozygosity for a mutation in *Brca1* on mammary tumor development in *Apc^{Min}/+* mice following irradiation.**
3. **To test for loss of heterozygosity at *Brca1* and *Apc* in mammary tumors arising in *Brca1* heterozygous *Min/+* mice.**

This approach represents an innovative use of these mouse model systems to investigate tumor development and the function of *Brca1*. If we can show an effect of heterozygosity for a mutation in *Brca1* on mammary tumor development in mice, we will then have a model in which to further investigate the function of *Brca1* in the mammary gland. In addition, these studies may have implications for the screening and treatment of women who carry a mutant allele of *BRCA1*.

BODY

I have listed below each of the tasks outlined in the Statement of Work from the original grant proposal. After each task, the progress made is described.

Task 1: To identify doses of irradiation that induce mammary tumor development in 129B6 *Apc*^{Min}/+ female mice. Months 1-12.

- Production of 129B6 female mice to use to develop the protocol for irradiation. Months 1-3, about 20 mice.
  Progress: We have produced the 129B6 F1 mice and used them to develop the protocol for irradiation. Prior to the use of any mice we determined designed and produced a foam frame that will hold the mice during irradiation. One concern was exposure of the ovaries to high doses of irradiation as we wanted the ovaries to remain functional after irradiation. Therefore, we located the position of the ovaries relative to the mammary fat pads and the position of the mammary gland epithelium at various ages. To do this, we anesthetized 3 45-day old 129B6 F1 mice, placed metal wound clips on each ovary and replaced the ovaries within the abdominal cavity. We then marked the position of the lymph node in each of the abdominal (4th) mammary fat pad. The lymph node can be used as a marker for mammary gland growth. We then placed the mice on their backs and x-rayed them to determine the relative position of the ovaries and mammary gland. We determined that the ovaries were sufficiently separated from the position of the leading edge of mammary gland growth in 40-day-old mice to allow shielding of the ovaries. We then constructed shields by bending small pieces of lead into a U-shape. We placed the shield over the abdomen of the mice and checked the placement on X-rays. To confirm that the shields would protect the ovaries from irradiation, we placed TLDs (dosimeters) under the mice in the approximate position of the ovaries, and TLDs under the mice in an unshielded region. We then exposed the mice to irradiation. We found that in female mice exposed to a dose of 500 cGy the shielded ovaries received a dose of less than 50 cGy. To test the function of ovaries of shielded mice as compared with unshielded mice, irradiated anesthetized 40 day old female mice them at doses of 100, 400, 500, 600 700, 800, and 900 cGy. The mice were then mated when they reached 60 days of age and the % of mice that became pregnant and carried a litter to term was determined. We tested 4 shielded mice and 4 unshielded mice at each dose. At all doses, 100% of the shielded mice became pregnant and carried the litter to term. Surprisingly, nearly all of the unshielded mice also successfully delivered litters. We concluded that the shielding offered sufficient protection to the ovaries.
- Production of 129B6 *Min/+* and +/+ female mice for irradiation experiments. Months 1-6. Mice for production, 50 129/SvEvTaconic females and 50 B6 *Min/+* males.
  Progress: complete
- Testing of 129B6 *Min/+* and +/+ mice. Months 3-10. Six doses (0, 50, 100, 200, and 500 cGy), 25 mice of each genotype/dose, 300 female mice.
  Progress: We generated and irradiated *Min/+* and +/+ mice at doses of 100, 300, 400, 500, 600, 700, 800, and 900 cGy (10 mice /dose). The dose range was changed from that proposed after we did a preliminary experiment where 9 *Min/+* and 12 +/+ mice were irradiated at 100 cGy. None of these mice developed any mammary tumors within 100 days after irradiation. To check for the presence of small mammary tumors, we collected the 1st and 4th mammary fat pads, stained them and examined the mammary glands for the presence of small tumors or hyperplastic lesions. Four of the nine mice had hyperplastic lesions in the mammary glands. This is in contrast to the effect of ENU, 100% of 129B6 F1 mice would have developed either tumors or hyperplasias by 100 days after treatment. We thus

decided that higher doses would most likely be needed to induce mammary tumors. Thus, the dose range was expanded. In order to get a faster reading on a reasonable dose range for the experiments, we tested small numbers of mice at each of the new doses. In this way, we would have an early read-out as to what dose range might be reasonable for the studies with the *Brca1/+* mice.

- Dissection of mice and analysis of data to generate dose response curve. Months 7-12.
  We have, to date, dissected 42 mice. The results are shown in Table 1 below. Even though the number of mice in each group is still small, there was not a large trend for increasing doses to yield an increased number of tumors. The remainder of the mice will be processed by the end of the summer. None of the +/+ mice developed any mammary tumors.

Table 1. Incidence of Mammary Tumors in Irradiated *Min/+* mice.

Dose (cGy)	# of mice	% with mammary tumors	% with mammary hyperplasia	%with any mammary lesion
100	9	0	44	44
300	5	20	60	60
400	3	67	33	67
500	7	0	29	29
600	3	67	33	100
700	7	14	42	57
800	2	50	50	100
900	6	50	17	67

Task 2. To determine the effect of heterozygosity for a mutation in *Brca1* on mammary tumor development in *Apc^{Min}*/+ mice following irradiation. Months 10-36.

- Production of 129B6 *Min/+ Brca1$^{\Delta 11/+}$* mice. Months 10-15. Mice needed for production; 50 129 *Brca1$^{\Delta 11/+}$* females and 50 B6 *Min/+* males.
  We have established the colony to produce the 129B6 mice segregating for *Min* and *Brca1*. To date, we have generated approximately 200 of the female mice for irradiation.
- Testing of 129B6 *Brca1$^{\Delta 11/+}$ Min/+* females. Months 12-21. Three doses (0, low and high) x 4 genotypes x 25 mice, 300 female mice.
  Based on the experiments described above, we chose to begin irradiating mice at 100 cGy (low dose) and 500 cGy (high dose). We have thus far irradiated 86 mice at 100 cGy and 96 mice at 500 cGy. We will analyze these mice and then decide whether we will test the effect of other doses.
- Dissection of mice and analysis of data. Months 18-23.
  To date, 46 of the 100 cGy and 34 of the 500 cGy mice have been dissected.
- Preparation of data for publication. Month 24-30.
  In process.
- Production of mice for further testing of doses. Months 18-24. Mice needed for production, 10-30 each 129 *Brca1$^{\Delta 11/+}$* females and B6 *Min/+* males.
  No progress.
- Testing of 129B6 *Brca1$^{\Delta 11/+}$ Min/+* mice at additional lower doses. Months 20-30. Mice needed, 25 mice of each genotype at each dose, 50-200 mice.
  No progress.

- Dissection of mice and analysis of data. Months 26-34.
  No progress.
- Preparation of data for publication and final report. Months 33-36.
  No progress.

Task 3. **To test for loss of heterozygosity at *Brca1* and *Apc* in mammary tumors arising in *Brca1* heterozygous *Min/+* mice.** Months

- Collection of tumors for analysis. Months 18-23 and 26-32. Mice same ones in Aim 2.
  As the mice are dissected, all mammary tumors are collected and processed for the LOH studies.
- Analysis of tumors for LOH at *Apc* and *Brca1*. Months 18-25 and 26-34.
  No progress.
- Preparation of data for publication. Months 26 and 35-36.
  No progress.

KEY RESEARCH ACCOMPLISHMENTS

- We have established a protocol for the irradiation of mice that preserves ovarian function, but allows exposure of all mammary glands of the mouse at the same dose.
- We have established two doses for the first testing of the susceptibility of *Brca1/+* mice to mammary tumor induction by irradiation.

REPORTABLE OUTCOMES

Oral Presentation:

None

Manuscripts:

None

CONCLUSIONS

We have found that 129B6 *Min/+* mice are susceptible to the induction of mammary tumors and hyperplasias after irradiation. None of the +/+ mice tested developed any mammary tumors or lesions. The data from dose response experiments has been used to begin the irradiation of 129B6 mice segregating for *Min* and a mutation in *Brca1*.

REFERENCES

none

APPENDICES

none